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THE AGEING AND DE-ALKYLATION OF ALKYL ALKYLPHOSPHONOCHOLINESTERASES [U]

BY

D.B. COULT & D.J. MARSH

CHEMICAL DEFENCE EXPERIMENTAL ESTABLISHMENT

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PORTON TECHNICAL PAPER NO: 864
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THE AGEING AND DE-ALKYLATION OF ALKYL ALKYLPHOSPHONO-CHOLINESTERASES

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SUMMARY

A study has been made of the de-alkylation and ageing of several alkyl methylphosphono-acetylcholinesterases. The rate of de-alkylation was found to vary with the structure of the alkyl group. Ageing, the conversion of the inhibited enzyme into a non-reactivatable form, was found to differ from de-alkylation in a number of instances, and showed a further variation with the oxime used as reactivator. This difference is attributed either to the inability to define ageing in precise chemical terms, or to the involvement of a two-step process.

The amount of enzyme activity restored by oximes was always less than that expected from the residual undo-alkylated enzyme.

The important factor in the failure of oxime therapy for nerve gas poisoning is considered to be the fraction of enzyme activity that can be restored under practical conditions. Comparison of rates of half-lives of ageing is not considered satisfactory.

The inability of oximes to restore an appreciable amount of the activity of pinacolyl methylphosphono-acetylcholinesterase (GD-inhibited enzyme) is attributed to its rapid de-alkylation.

Possible means of obtaining improved reactivation of inhibited cholinesterases are indicated.

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Supt., Chemistry Research Division.

(Sgd). A.S.C. Hill,
Deputy Director.

DBC/DJM/GC

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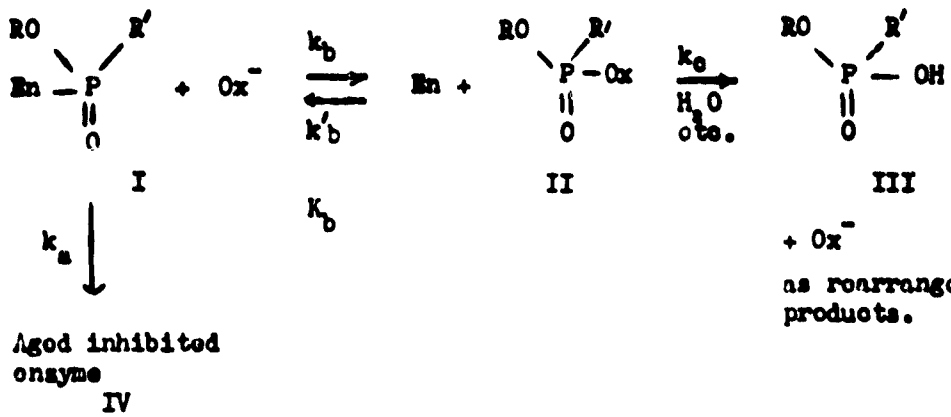
INTRODUCTION

Following the discovery that poisoning by some nerve gases is resistant to treatment with oximes, considerable effort has been devoted to the study of the processes involved in the reactivation of inhibited cholinesterases. These were represented by a series of reactions (1), which in a more simplified form are:-



Where En = enzyme (ChE) and Ox = oxime.

Results previously reported have shown that neither the values of the equilibrium constant K_b (1), or of k_c (2), vary greatly when the ester group RO is varied.

It has been suggested that rapid ageing, the conversion of the inhibited enzyme to a non-reactivatable form, is the cause of the resistance of poisoning by GD to oxime therapy (3). This was based on the finding that human red cell acetylcholinesterase inhibited by GD could be reactivated to a small extent at pH 9, but not at all at physiological pH. Since it was known that the rate of ageing of other enzymes increased with decrease in pH, it was inferred that ageing of the GD-inhibited enzyme was extremely rapid at physiological pH. This small amount of reactivation of the GD-inhibited enzyme at pH 9 can alternatively be accounted for by the increased rate of breakdown of the phosphonylated oxime, II, due to base catalysis (2), so that reactivation is favoured. Further evidence that the ageing of GD-inhibited enzyme is rapid was recently obtained (1), when, in experiments with bovine erythrocyte acetyl-cholinesterase, a small amount of reactivation was obtained initially, but the amount of free enzyme liberated subsequently decreased.

It seemed therefore that rapid ageing is responsible for the resistance of GD poisoning to oxime therapy, but the evidence for this was indirect, being based on measurements in which oximes were used to restore the activity of "unaged" inhibited enzyme. This was regarded as unsatisfactory, since it has been shown that the reaction of oxime with inhibited enzyme is a reversible process (1) and that consequently the amount of enzyme activity restored depends on the oxime concentration used. A more satisfactory demonstration of the rapid change of the inhibited enzyme to a non-reactivatable form, preferably without the use of oximes, was therefore considered necessary.

In the case of DFP-inhibited horse-serum pseudocholinesterase, Berends et al. (4) showed that ageing could be correlated with the loss of an iso-propyl group from the di-isopropyl phosphoryl enzyme. Their technique for following the de-alkylation process was not, however, suitable for studies of the process where it is rapid. An improved technique has therefore been developed, details of which have been reported (5). Using this technique, studies of the de-alkylation of a series of alkyl methylphosphono-acetyl cholinesterases have been made, together with ageing measurements carried out under the same conditions of pH and temperature.

EXPERIMENTAL

(a) Materials

The enzyme preparation used was Bovine Erythrocyte Acetyl-cholinesterase (Winthrop Laboratories Inc.). A check was made on the purity of the two batches used. Details of the method and results are given in the Appendix.

The purity of the ^{32}P-labelled inhibitors used in the de-alkylation studies was at least 95 % and that of other inhibitors used in the ageing studies at least 98 %. All analyses were carried out by the Schoneemann method (6).

(b) **De-alkylation Studies**

All de-alkylation measurements were made using the technique recently described (5).

(c) **Ageing Studies**

Ageing was measured by a technique similar to that used by Berry and Davies (3).

1 ml aliquots of diluted enzyme preparation containing 100 units/ml (4×10^{-9} M) were inhibited with 10^{-7} M inhibitor and allowed to stand at 25°C in a thermostat bath. At suitable time intervals, samples were removed and 1 ml of 10^{-2}M oxime solution added, and reactivation allowed to proceed. The time allowed for reactivation was usually 30 min, except for GD-inhibited enzyme where 5 and 1 min only were allowed for the oximes P2S and TMB4 respectively (vide infra). At the end of this period, 1 ml of acetylcholine solution (10% w/v) was added and the mixture diluted to 25 ml with 0.9% saline solution. The rate of acid production at pH 7.4 and 25°C was followed using the Radiometer Titrigraph SBR2. This rate was used as a measure of enzyme activity.

RESULTS

(a) **De-alkylation**

The kinetics of the de-alkylation process for phosphorylated enzymes has not previously been established, but it was found that the data of Berends et al. (4) for DFP-inhibited pseudocholinesterase followed first order kinetics. The present data show some degree of scatter, but give reasonable first order plots (Figures 1 - 5) from which rate constants were evaluated (see Table 1). When the data were plotted in the forms for second order or autocatalytic reactions, pronounced curves were obtained.

Two batches of enzyme were used, and although it was found that neither underwent unspecific phosphorylation as shown by the method developed previously (5), a difference became apparent in the de-alkylation studies. With the first sample, 100% de-alkylation of the inhibited enzyme occurred if it was left for an extended period, as shown by the recovery of 100% methylphosphonic acid and no alkyl hydrogen methylphosphonate. With the second sample however, only 85% de-alkylation occurred, irrespective of how long the inhibited enzyme was allowed to stand, and the result was the same with each of the different inhibitors used. This behaviour is attributed to the binding of 15% of phosphorus in some unspecific way, i.e. not at the active centre, so that it does not undergo de-alkylation. Results of experiments carried out with this batch of enzyme have been corrected accordingly, and where the same

TABLE 1

RATE CONSTANTS FOR AGEING AND DE-ALKYLATION OF ALKYL METHYLPHOSPHONYLATED ChE AT pH 7.4 AND 25°C

R	Corresponding G agent	Ageing † (Reactivation time 30 min)		Dealkylation	
		Oxime used	k (min^{-1})	k (min^{-1})	Half-life (min)
Propyl-2	GB	P2S TMB4 MINA	See Figure 6 See Figure 6 0.00077	0.0013	550
Butyl-2	T2132	P2S TMB4 MINA	0.0077 0.0073 0.0080	0.0082	85
3-Methyl-butyl-2	T2137	P2S TMB4 MINA	0.019 0.012 See Figure 7	0.012	58
3,3-Dimethyl-butyl-2 (Pinacolyl)	GD	P2S TMB4 MINA	• 0.20 ʃ 0.13 No reactivation	0.115	6.0
Cyclohexyl	GF	P2S TMB4 MINA	0.0023 See Figure 8 See Figure 8	0.00017	4000

• Reactivation Time - 5 min.

ʃ Reactivation Time - 1 min.

† Half-lives for ageing are not given owing to possible misinterpretation (see discussion).

TABLE 2

PERCENTAGE REACTIVATION OBTAINED USING 5×10^{-3} OXIME AT pH 7.4 AND 25° C FOR 30 MIN

RO, Me, P, O, ChE — R	Corresponding G agent	Oxime	% Enzyme Reactivated † at zero time of Ageing	Maximum % Enzyme Reactivated (if different)
Propyl-2	GB	P2S TMB4 MINA	43.5 37.5 35.0	- - -
Butyl-2	T2132	P2S TMB4 MINA	27.5 27.0 27.5	- - -
3-Methyl butyl-2	T2137	P2S TMB4 MINA	19.0 13.5 7.0	- - 14.0
3,3-Dimethyl butyl-2 (Pinacolyl)	GD	P2S TMB4 MINA	19.0 • 12.5 ∮ -	- - -
Cyclohexyl	GF	P2S TMB4 MINA	25 6.5 8.5	- 21.0 27.5

† Extrapolated values.

∮ After 1 min reactivation.

• After 5 min reactivation.

inhibitor was used with both batches of enzyme the data were in good agreement.

(b) Ageing

Ageing has been shown previously to be a first order process (7, 8, 9). Data obtained in some cases in the present study also appear to conform to first order kinetics, and rate constants were obtained graphically where possible. (See Table 1 and Figures 1 - 5). In other cases, however, the ageing process was found to be less straightforward as shown in Figures 6 - 8 and no evaluation of rate constants was possible. It may be noted further that in most cases the amount of free enzyme obtained was small, although a large excess of oxime was used. An initial figure for each case was obtained by extrapolating plots of the percentage reactivated enzyme back to zero time. The figures obtained are shown in Table 2. To obtain rate constants for ageing, it must be assumed that these amounts of enzyme reactivated are directly related to the amount of residual reactivatable enzyme. This point will be discussed later.

TABLE 1

EFFECT OF OXIME* ON THE DE-ALKYLATION OF CYCLOHEXYL METHYLPHOSPHONYL ChE

Time of De-alkylation (hr)	% De-alkylation (in absence of oxime)	% De-alkylation (in presence of oxime)
8	21	20
16	36	39
24	39	25

* P2S to give a concentration 5×10^{-3} M was added half an hour before the measurement of de-alkylation in each case.

DISCUSSION

The results of the study of the de-alkylation of a series of alkyl methylphosphono-acetylcholinesterases show that there are large differences in the rates for different compounds. The results also show that rates of ageing, where it has been possible to measure them, do not always agree with the rates of de-alkylation for the same inhibited enzyme. The implications of these findings will be discussed under two headings, viz. the mechanism of ageing and practical aspects in relation to oxime treatment for nerve gas poisoning.

(a) The mechanism of ageing

It had been anticipated, following the finding of the Dutch workers (4) for the case of DFP-inhibited pseudocholinesterase, that ageing, defined as the change in the inhibited enzyme to a form that could no longer be reactivated with oximes, could be correlated with de-alkylation, the loss of an alkyl group from the phosphonyl moiety attached to the enzyme. Examination of these results shows this not to be so in all cases. The agreement in the rates is good for the 2-butyl compound, and for the 3-methyl butyl-2 and pinacolyl compounds where TMB-4 was used as reactivator. The difference in the rates of the two processes is most marked for the cyclohexyl derivative.

The results for the GB-inhibited enzyme are anomalous since, unlike those for the other compounds, they indicate that ageing is a slower process than de-alkylation. This is clearly absurd, since de-alkylated enzyme cannot be reactivated, as was demonstrated by Berends et al. (4).

The percentage of enzyme activity restored was, however, always less than that which would be expected if all the inhibited enzyme, not shown by the other technique to be de-alkylated, were reactivated. This was true for all the inhibited enzymes studied, and in no case was the figure obtained by extrapolation to zero time anywhere near 100 % (see Table 2).

These considerations explain the anomalous rates of ageing for GB-inhibited enzyme, which are clearly liable to mis-interpretation.

In some of the other cases, ageing is apparently a more rapid process than de-alkylation, and also the rate of ageing varied when different oximes were used as reactivators. Three possible explanations for these findings can be advanced:-

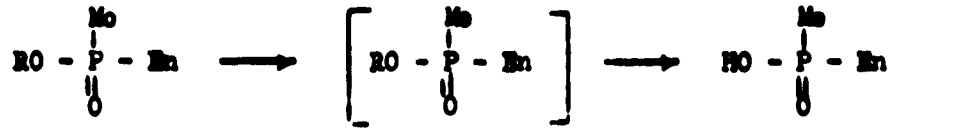
(i) The usual definition of ageing, conversion of the inhibited enzyme into a non-reactivatable form is invalid. Ageing is measured in terms of the amount of enzyme activity restored by reactivators (usually oximes), but it does not follow that this is a true measure of residual reactivatable inhibited enzyme (I). This will only be the case if either this restoration is complete, or if the amount of activity restored is directly proportional to the amount of I.

The former may be achieved where the ageing step is either very slow compared with the other steps or where, as was found by Berends et al. (4) oxime terminates the ageing process. With the compounds studied it was clear that the activity of all the inhibited enzyme, shown by the other method to be de-alkylated was not restored, and also it did not appear that oxime terminated ageing. In particular, with the pinacolyl compound, no reactivation was found to occur when the inhibited enzyme was allowed to stand with P2S or TMB4 for 30 minutes, the same time as with the other compounds, but, if the time was reduced to 5 minutes and 1 minute respectively, then some enzyme activity was restored, showing that ageing proceeds in the presence of oxime, at least in this case.

The alternative, that the amount of activity restored is directly proportional to the amount of residual reactivatable inhibited enzyme is considered unlikely in view of the complexity of the situation. Restoration of activity does not depend simply on the reaction of oxime with the inhibited enzyme, but, as first shown by Wilson and Ginsburg (10) and later by Scaife (11), an equilibrium may be set up. This equilibrium, for the inhibited enzymes and oximes used in the present investigation, is greatly in favour of inhibited enzyme and moreover, may only be set up momentarily, owing to the occurrence of the other reactions shown in the scheme on page 1 (1).

It is concluded, therefore, that ageing as defined is not necessarily measured by the amount of activity restored by reactivators. This provides a possible explanation of the observed results, which, however, appears inadequate to account for the large difference in the rates of ageing and de-alkylation in the case of the GF-inhibited enzyme.

(ii) An alternative explanation of the results is that a two-stage process is involved, which might be represented:-



Inhibited Enzyme I | Oxime Resistant Enzyme IV | De-alkylated inhibited enzyme V

Some change in the conformation of the inhibited enzyme may occur, such that the approach of the oxime to the phosphonyl group or the attachment of its quaternary group to the anionic site of the enzyme, is not favoured. This would give rise to "oxime resistance", preceding de-alkylation, the extent of which would depend on the structure of both the oxime and the inhibiting alkyl methylphosphonyl- group. It is possible that in different cases either or both of the inhibited enzymes I and IV above can be reactivated, depending on the conditions and the oxime used. The shapes of the curves obtained for the amount of reactivatable enzyme at different times for enzyme inhibited by GB (Figure 6), T2137 (Figure 7) and GF (Figure 8) are consistent with this possibility as they can be regarded as the result of the combination of theoretical curves for the concentrations of the initial reactant and intermediate in a two-stage process, an example of which is shown in Figure 9.

A change in the conformation of the inhibited enzyme could also facilitate S_N1 fission of the alkyl group on the phosphorus by bringing it closer to the anionic site.

(iii) A further possibility is that de-alkylation proceeds at a faster rate in the presence of oxime. This is difficult to test, but P2S was added to GF-inhibited cholinesterase which had stood for different intervals and after a further 30 minutes the extent of de-alkylation was measured. The results were not significantly different from those in cases where the inhibited enzyme had stood for the same total time in the absence of P2S. The experiments are not very conclusive, but indicate no effect of oxime on the rate of de-alkylation.

It is not yet possible to decide in favour of either explanation (i) or (ii) above. It is possible that both are valid.

(b) Practical Aspects

As noted above, the rate of de-alkylation of alkyl methylphosphono-acetylcholinesterases varies with the structure of the alkyl group. Ageing, as measured by the amount of enzyme activity that can be restored, may not be the same as de-alkylation and may vary with the oxime used.

The use of rates of ageing as a criterion is misleading. It is implied that complete reactivation can be obtained at zero time, whereas the actual amount of activity restored is nowhere near 100 % as can be seen from the extrapolated figures given in Table 2. It is even more misleading to quote a half-life for ageing, since this gives the impression that 50 % of the inhibited enzyme can be reactivated at this time. This is clearly not the case, and it is considered that the important factor is the actual amount of enzyme activity that can be restored.

De-alkylation of pinacolyl methylphosphono-acetyl cholinesterase (the GD-inhibited enzyme) is rapid. Poisoning by GD is very resistant to treatment (12), and this problem will now be discussed.

In earlier studies (3), no reactivation at physiological pH of GD-inhibited human red blood cell acetylcholinesterase could be achieved, whereas some reactivation has now been obtained immediately after inhibition by measuring the restored enzyme activity shortly after addition of oxime. The earlier failure to restore any activity can be attributed to the continuing de-alkylation, presumably to completion, which occurred during the 30 minutes allowed for reactivation.

In the present experiments, the maximum amount of enzyme activity restored from GD-inhibited bovine erythrocyte acetylcholinesterase at pH 7.4 and 25° C was about 15 % of the original activity. This was obtained by using 5×10^{-3} M P2S, added 1 minute after inhibition, and measuring the enzyme activity at the optimum time (5 min). This concentration of oxime is unrealistically high and it is considered that, with the level of oxime that could be used therapeutically, the amount of enzyme activity restored would be very small.

It is clear that this inability to restore the activity of a significant amount of GD-inhibited cholinesterase, due to rapid de-alkylation, is the basis of the resistance of poisoning by GD to oxime therapy. The present investigation has not provided any obvious lead to an improved therapy for nerve gas poisoning, but consideration of the results, and those of earlier studies (1, 2) enables the following suggestions, not necessarily original, to be put forward:-

(i) A less toxic reactivator than say, TMB4, is desirable since higher concentrations could be used, so favouring the forward reaction (increasing k_b) to give more free enzyme.

(ii) A reactivator, whose phosphonylated derivative undergoes rapid breakdown, ideally with the regeneration of the reactivator, would be advantageous since removal of the phosphonylated derivative will drive the reaction sequence (page 1) to the right, i.e. reactivation will be favoured.

(iii) In addition to, or in place of, (ii) catalysts to increase the rate of breakdown of the phosphonylated derivative should be sought, so that again reactivation would be favoured. Catalysis of this breakdown by bases and by cobaltous ion has been demonstrated (1).

(iv) Reactivators, capable of removing the de-alkylated phosphonyl group from the enzyme should be sought.

CONCLUSIONS

De-alkylation of alkyl methylphosphono-acetylcholinesterases occurs, the rate varying with the structure of the alkyl group. Ageing is not the same as de-alkylation in a number of instances, and shows a further variation with the oxime used as reactivator. This is due to either the inability to define ageing in precise chemical terms, or to the involvement of a two-stage process.

The amount of enzyme activity restored is always less than that expected from the residual unde-alkylated enzyme.

The important factor in the failure of oxime therapy for nerve gas poisoning is the fraction of enzyme activity that can be restored under practical conditions. Comparison of rates or half-lives of ageing is unsatisfactory.

Rapid de-alkylation of pinacolyl methylphosphono-acetylcholinesterase (GD-inhibited enzyme) occurs and this accounts for the inability of oximes to restore an appreciable amount of its activity.

ACKNOWLEDGEMENTS

Miss W.A. Searle and W.G. Wills gave considerable assistance with the de-alkylation measurements. J.P. Rutland assessed the purity of the samples of acetylcholinesterase used.

The ^{32}P labelled inhibitors used were received from Suffield Experimental Station, Canada, or prepared by R. Brown and P. Rich from intermediates supplied by Suffield.

Valuable discussions with Dr. G. Read*, in collaboration with whom this investigation was commenced, are acknowledged.

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Deputy Director.

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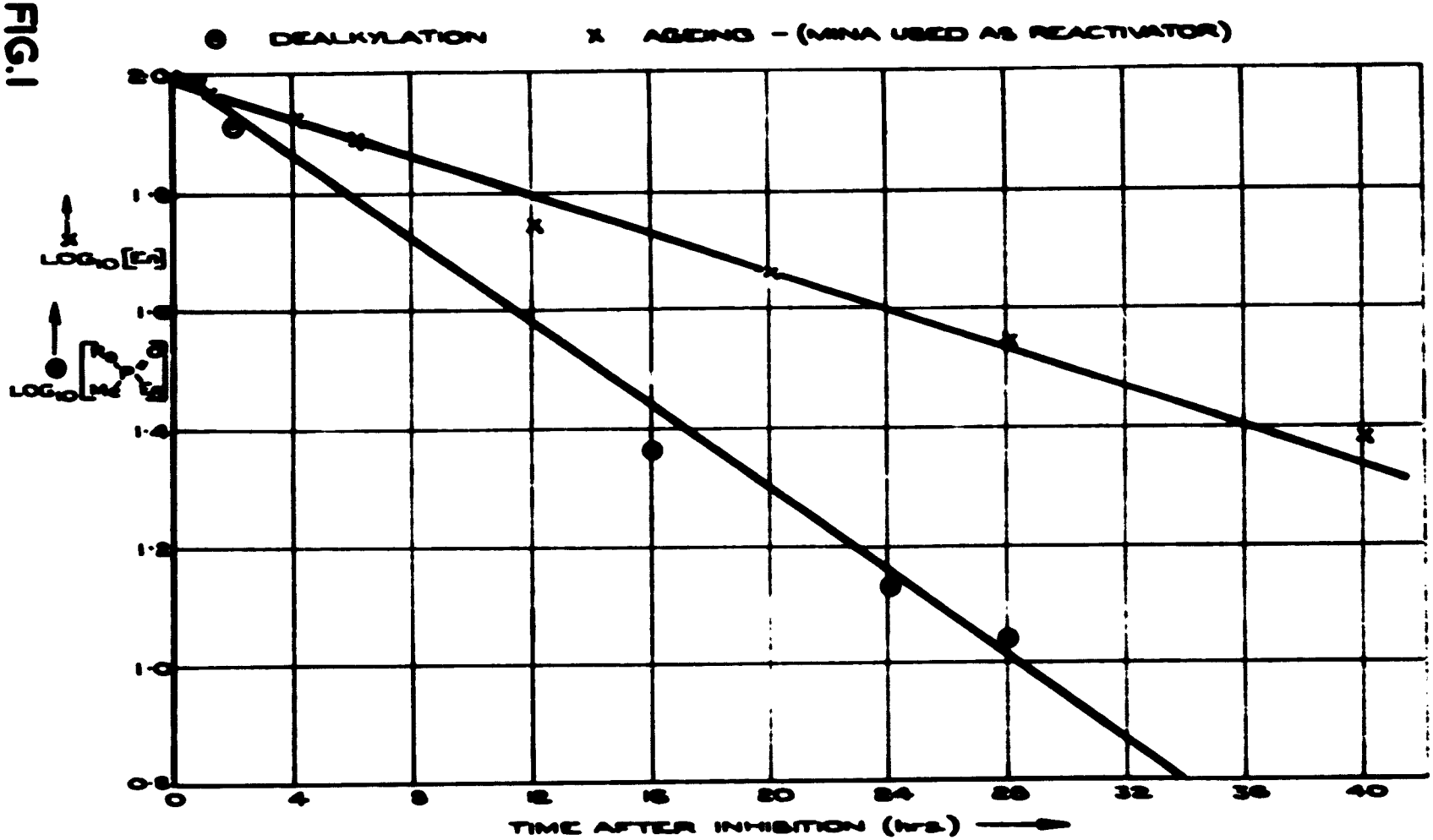
REFERENCES

(1)	D.B. Coult and D.J. Marsh.	P.T.P.863.
(2)	D.B. Coult.	P.T.P.862.
(3)	W.K. Berry and D.R. Davies.	P.T.P.783.
(4)	F. Berends, C.H. Posthumus, I.v.d. Sluys and F.A. Doierkauf.	Biochim.Biophys.Acta, 1959, 34, 576.
(5)	D.B. Coult, D.J. Marsh and G. Read.	P.T.P.834.
(6)	D.J. Marsh and E. Nealo.	Chemistry and Industry, 1956, 494.
(7)	F. Hobbiger.	Brit.J.Pharmacol., 1955, 10, 356; 1956, 11, 295; 1957, 12, 438.
(8)	I.B. Wilson.	Disc.Faraday Soc., 1955, 20, 119.
(9)	D.R. Davies and A.L. Green.	Biochem.J., 1956, 63, 529.
(10)	I.B. Wilson and S. Ginsburg.	Biochim.Biophys.Acta, 1958, 18, 169.
(11)	J.F. Scaife.	Can.J.Biochem.Physiol., 1959, 37, 1307.
(12)	W.K. Berry, D.R. Davies and A.L. Green.	P.T.P.751.

FIG.1



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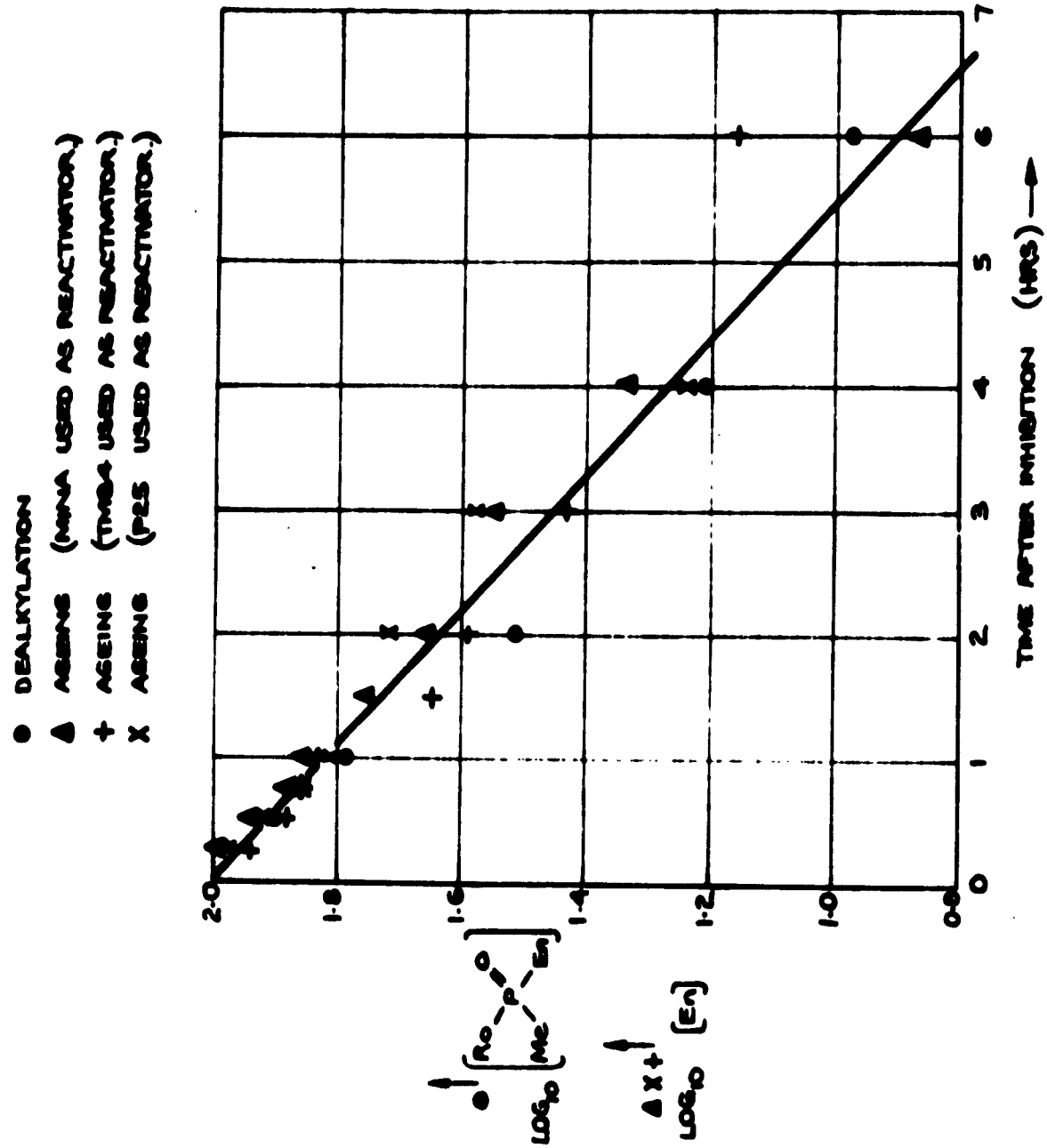
PT457

COMPARISON OF THE 'AGEING' AND 'DEALKYLATION' OF GB-INHIBITED ChE.



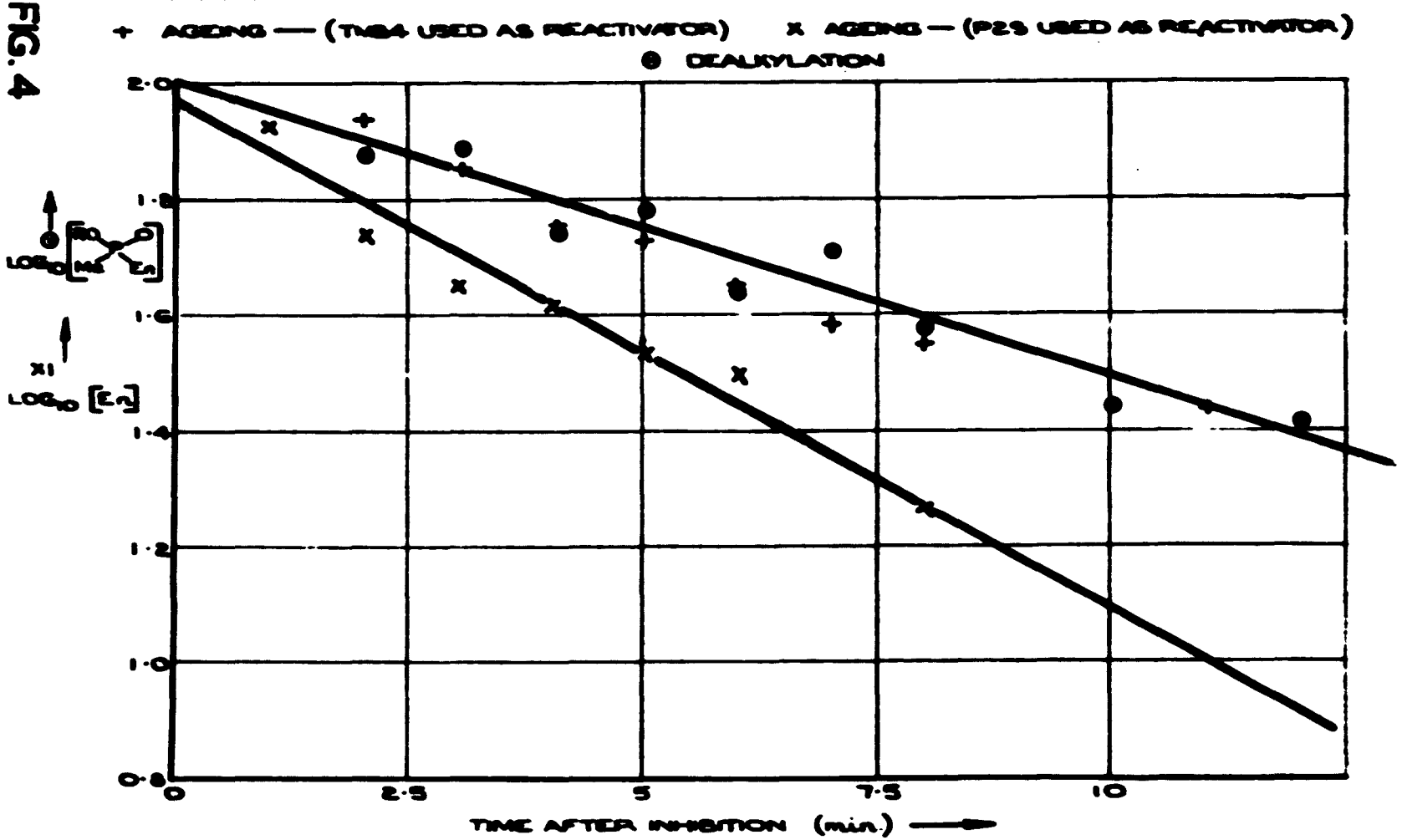
COMPARISON OF THE 'AGEING' AND 'DEALKYLATION' OF T2132-INHIBITED ChE.

FIG.2.

FIG. 3.

COMPARISON OF THE 'AGEING' AND 'DEALKYLATION' OF T2137-INHIBITED ChE.

FIG. 4



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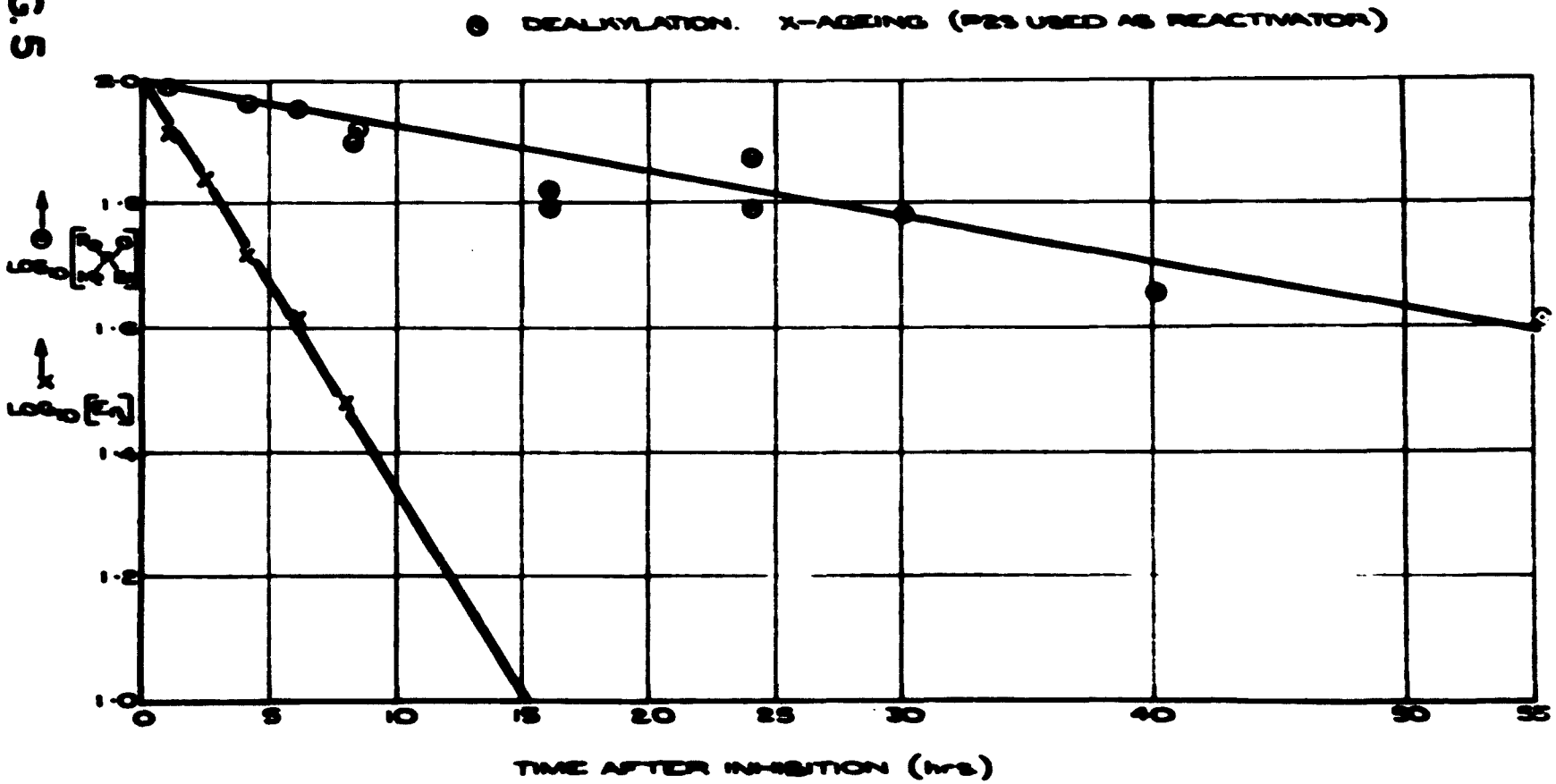
COMPARISON OF THE 'AGEING' AND DEALKYLATION OF GD-INHIBITED ChE.

FIG. 5



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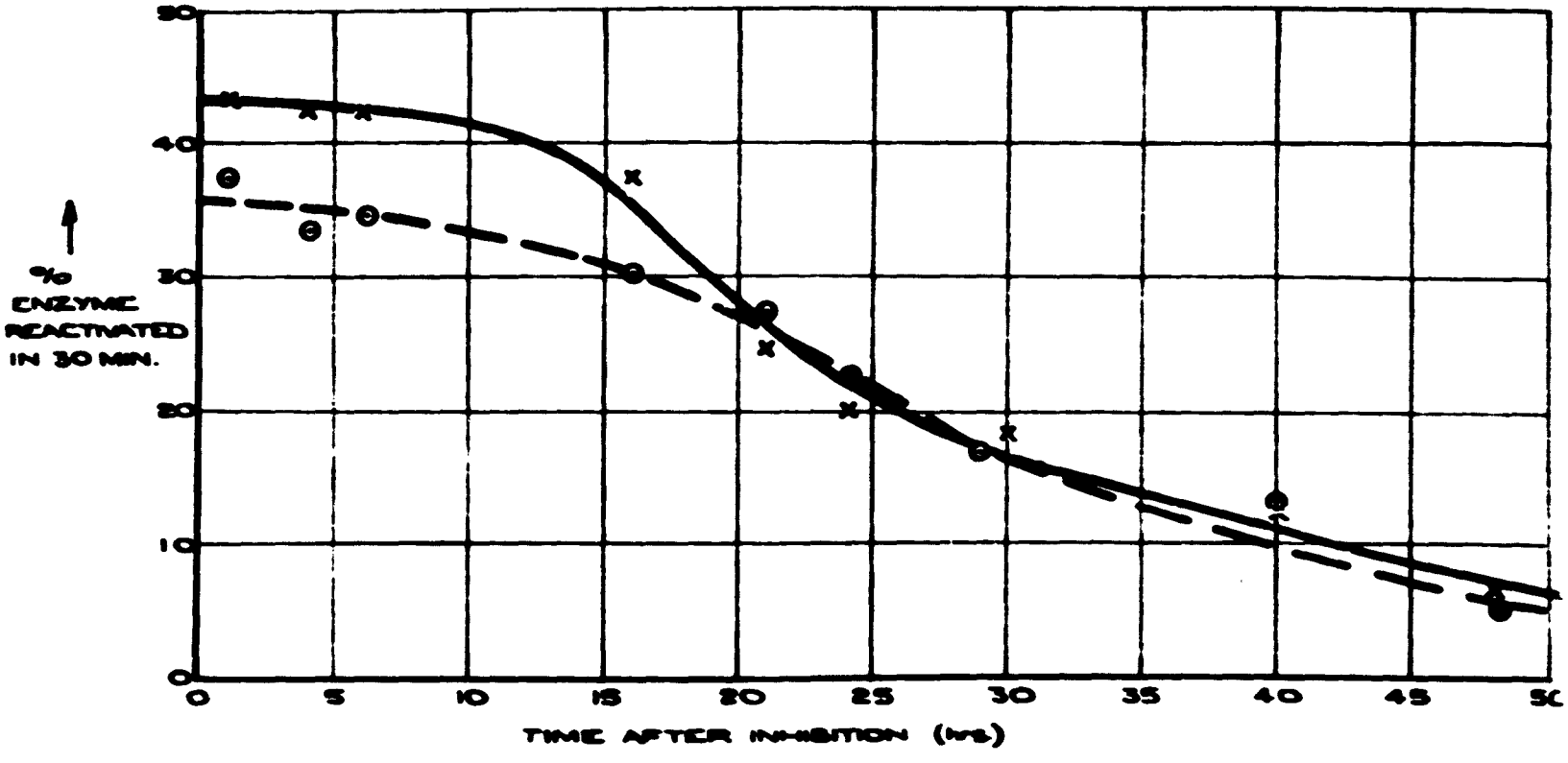
COMPARISON OF THE 'AGEING' AND DEALKYLATION OF GF-INHIBITED ChE.

FIG. 6.



REACTIVATION OF GB-ChE (AGED AT 25° C AND pH 7·4) BY 5×10^{-3} M TMB4, ⊙ AND BY 5×10^{-3} M P2S X

FIG. 7.

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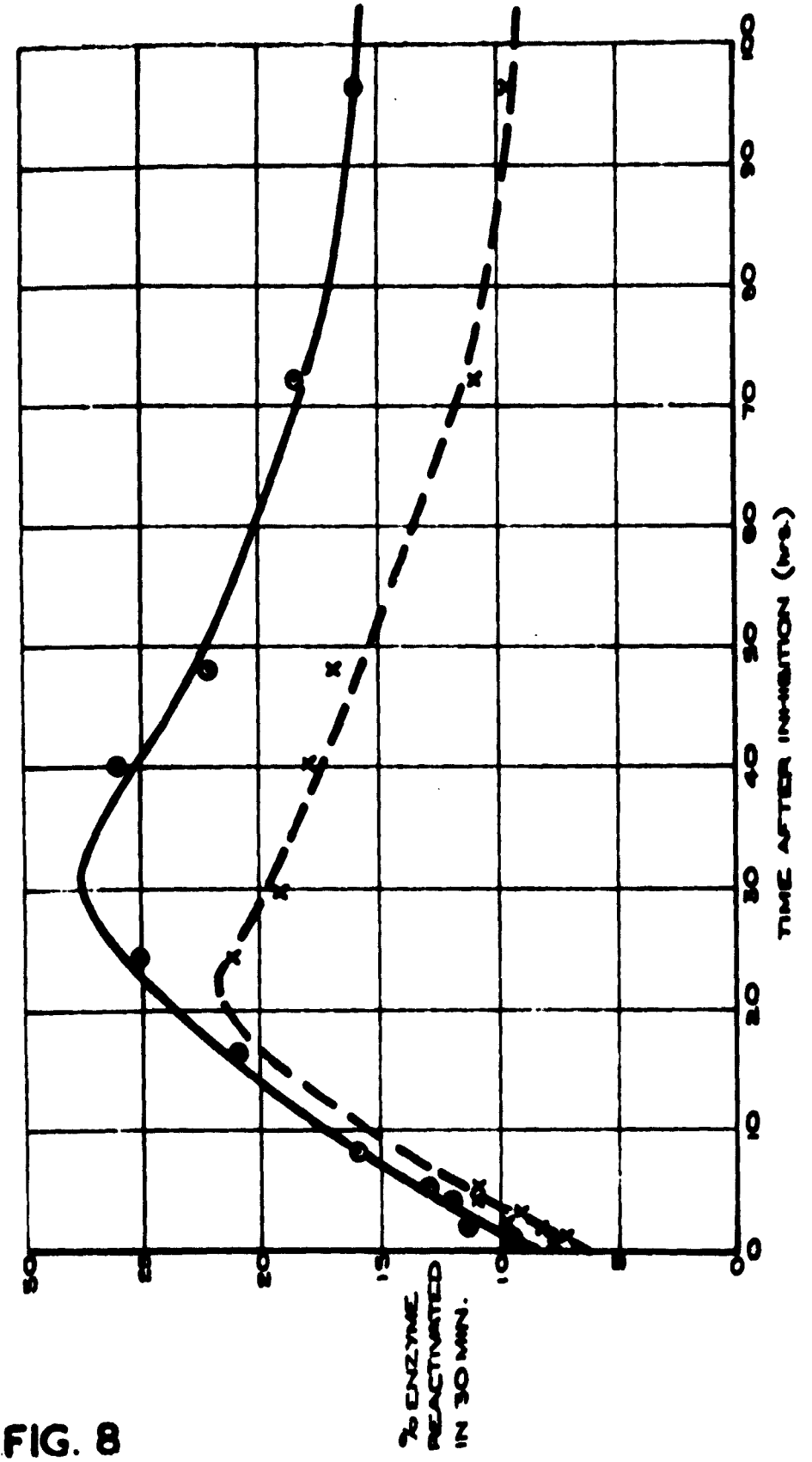
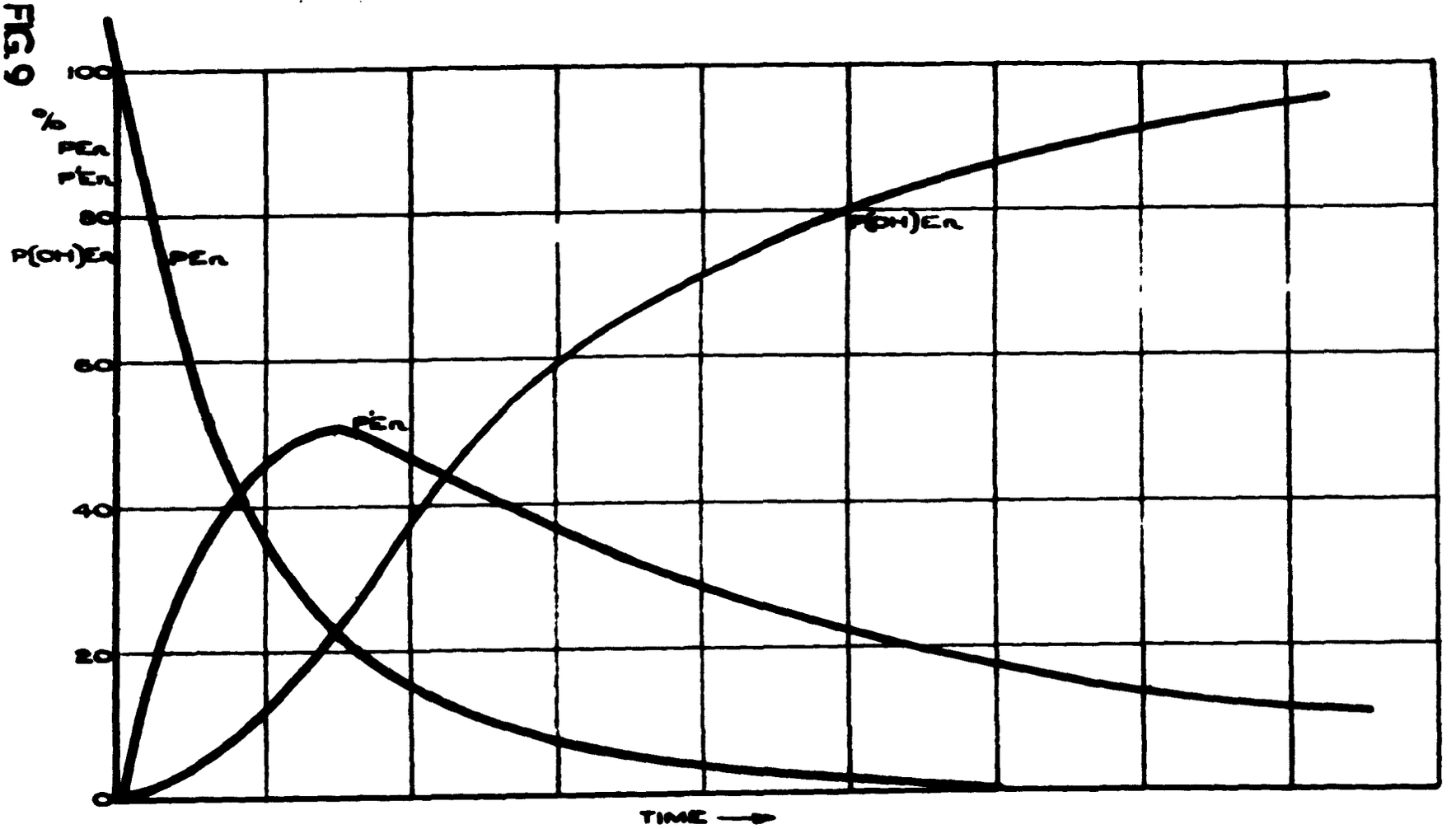


FIG. 8

REACTIVATION OF GF-ChE ['AGED' AT 25°C AND pH 7·4] BY 5×10^{-3} M TMB4, X AND BY 5×10^{-3} M MMA O

FIG. 9



THEORETICAL PLOTS OF THE PERCENTAGE OF PEn, P'En AND P(OH)En AGAINST TIME FOR THE REACTIONS :-

PEn $\xrightarrow{k_1}$ P'En $\xrightarrow{k_2}$ P(OH)En ; ASSUMING $k_1 = 2k_2$

PORTON TECHNICAL PAPER 864

PT436

APPENDIX TO P.T.P. 864

PURITY OF THE WINTHROP BOVINE ERYTHROCYTE ACETYLCHOLINESTERASE

An aqueous solution of the Winthrop Bovine Erythrocyte acetylcholinesterase was examined for the presence of pseudo-cholinesterase, arylesterase and aliesterase. The activity of the solution towards Acetylcholine (AcCh), Butyrylcholine (BuCh), Phenylacetate (PhAc) and glyceryl tri-butyrate (TB) was tested. The effect of 10^{-3} M eserine on the hydrolyses was also followed.

Results are shown in Table 4.

TABLE 4

HYDROLYSIS OF SOME SUBSTRATES BY WINTHROP BOVINE ERYTHROCYTE ACETYLCHOLINESTERASE

Substrate	Hydrolysis (μl/CO$_2$/0.5 ml (dilu. 1 in 10)/30 min)							
	Control		Inhibited		% Inhibition		AcCh = 100	
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 1	Batch 2	Batch 1	Batch 2
AcCh	54	69	2	1	96	98	100	100
BuCh	No significant hydrolysis							
PhAc	16	18	6	7	39	37	24	26
TB	4	4	5	5	0	0	6	6

The results indicate that the preparations did not contain any significant amounts of other esterases.

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